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ACCELERATED COMMUNICATION

Paclitaxel (Taxol) Inhibits Protein Isoprenylation and Induces Apoptosis in PC-3 Human Prostate Cancer Cells

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SUMMARY

Paclitaxel was examined for its effects on cell survival, internucleosomal DNA fragmentation, and protein isoprenylation in the human prostate cancer cell line PC-3. Treatment of cells with paclitaxel at 5–60 nM for 24 hr resulted in a dose-dependent inhibition of cell viability (IC_{50}, 31.2 nM), which was partially prevented by supplementing the cell culture medium with two nonsterol polyisoprenyl compounds, farnesyl-pyrophosphate (-PP) and geranylgeranyl-PP (3 μM each). Furthermore, agarose gel electrophoresis of DNA extracted from cells treated with paclitaxel (15–60 nM) for 24 hr showed DNA laddering with production of fragments of 180-base pair multiples, indicating the occurrence of apoptotic cell death. Internucleosomal DNA fragmentation by paclitaxel was also detected by a photometric enzyme immunoassay using antihistone antibodies; if culture medium was supplemented with farnesyl-PP and geranylgeranyl-PP (3 μM each), a reduction in mono- and oligoucleosome production was observed. The post-translational incorporation of metabolites of (*RS*)-[5-^{3}H]mevalonolactone (100 μCi/ml) into prenylated proteins of PC-3 cells was inhibited by paclitaxel at 30 and 60 nM. In addition, the immunoprecipitation of p21ras and p21rap-1 proteins from PC-3 cells exposed to paclitaxel (30 and 60 nM) and labeled with (*RS*)-[5-^{3}H]mevalonolactone showed a substantial inhibition of the incorporation of farnesyl and geranylgeranyl prenoid groups, respectively, into the aforementioned proteins. These results indicate that the inhibition of protein isoprenylation is a novel component of the complex biochemical effects of the drug and plays an important role in the mechanism of paclitaxel cytotoxicity in PC-3 cells.

Eukaryotic polypeptides that are initially synthesized with the carboxyl-terminal amino acid sequence CAAX, including a variety of signal-transducing proteins such as G proteins and cGMP phosphodiesterases, can be targeted for a series of sequential post-translational modifications (1). This novel processing pathway includes the isoprenylation of the cysteine residue with a C_{15} farnesyl or C_{20} geranylgeranyl moiety, followed by proteolysis of the three terminal residues and α-carboxyl methyl esterification of the cysteine residue (2). The isoprenoid farnesyl-PP is a particularly important intermediate in the mevalonate pathway. It is used to synthesize cholesterol (3), and it is also bound covalently to the proteins encoded by the *ras* oncogenes (4), whose mutated forms are among the most common genetic abnormalities in human cancers (5). In addition, *ras*-related, low molecular weight G proteins, including the products of the *rap*-1, *rab*, and *rho* genes, have been shown to be geranylgeranylated (1). Thus, isoprenylation is a critical step for subcellular localization of and acquisition of biological activity by signal-transducing proteins that play a pivotal role in cell growth regulation.

Inhibitors of the enzyme HMG-CoA reductase, such as lovastatin, block the production of mevalonate and its metabolites, including farnesyl-PP and geranylgeranyl-PP, and have been shown to suppress the proliferation of many cell types (6). Inhibition of isoprenoid biosynthesis by lovastatin triggers apoptosis in the human promyelocytic cell line HL-60 (7), an effect that is also produced by paclitaxel in the same cell line (8). Paclitaxel is a terpene compound obtained from the bark of *Taxus brevifolia* and is characterized by strong affinity for tubulin protein and remarkable antitumor activity *in vitro* and *in vivo* (9). Apart from its well known antimicrotubular effect, other pharmacodynamic properties of the drug are still to be examined. In the present study, the effects of paclitaxel on apoptosis and protein prenylation were investigated in the human prostate cancer cell line PC-3.

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ABBREVIATIONS: PP, pyrophosphate; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; bp, base pair(s); MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate.

Materials and Methods

Reagents and drugs. The following supplies were obtained from the designated sources: 123-bp DNA ladder, RPMI 1640 medium, heat-inactivated fetal bovine serum, and L-glutamine for cell culture (GIBCO-BRL, Gaithersburg, MD), tissue culture plasticware (Falcon, Boston, MA), and (*RS*)-[5-^{3}H]mevalonolactone (50–60 Ci/mmol), farnesyl-PP, and geranylgeranyl-PP (American Radiolabeled Chemicals, St. Louis, MO). Nonidet P-40 and protease inhibitors were obtained from ICN Biomedical (Irvine, CA), anti-v-Ha-*ras* rat monoclonal antibody clone Y13–259 was purchased from Oncogene Science (Uniondale, NY), and anti-*rap*-1/K-*rev*-1 rabbit polyclonal antibody clone 121 was from Santa Cruz Biotechnology (Santa Cruz, CA). The Enlightning autoradiography enhancer was obtained from NEN-DuPont (Boston, MA), the bicinchoninic acid protein assay reagent kit was purchased from Pierce (Rockford, IL), GammaBind Plus Sepharose was from Pharmacia Biotech (Uppsala, Sweden), proteinase K, glycogen, Coomassie blue G-250, and bovine pancreatic RNase A were obtained from Boehringer Mannheim (Indianapolis, IN), and silver stain reagents were from Bio-Rad (Richmond, CA). Lovastatin, a competitive inhibitor of HMG-CoA reductase, was from Biomol (Plymouth Meeting, PA); the lactone form was converted to the sodium salt as described previously (10), and a 10 mM solution was stored at −20°. Paclitaxel was obtained through the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD), and a 10 μM stock solution was prepared in dimethylsulfoxide. Other chemicals were from Sigma Chemical Co. (St. Louis, MO).

Cell line and culture conditions. The human androgen-independent prostate cancer cell line PC-3 (American Type Culture Collection, Rockville, MD) was maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum and 2 mM L-glutamine. Cells were routinely subcultivated when 75% confluent, at a ratio of 1:15, by treatment with 1.5 mM EDTA and were plated in 75-cm^2 culture flasks. Cells were incubated in an atmosphere of 5% CO_2/95% air at 37°.

Assay of paclitaxel cytotoxicity. PC-3 cell proliferation was evaluated by using the CellTiter 96 AQueous cell proliferation assay kit (Promega, Madison, WI). This system is based on the tetrazolium compound MTS, which in the presence of the electron-coupling agent phenazine methosulfate is reduced by living cells to yield a soluble formazan product that can be assayed colorimetrically (11). Cells were routinely seeded in 96-well sterile plates, at a density of 1500 cells/well in 100 μl of medium, and were incubated for 24 hr. Paclitaxel was added to a final concentration of 5–60 nM, in a volume of 150 μl/well. Twenty-four hours later, 30 μl of freshly prepared, combined, phenazine methosulfate/MTS solution were pipetted into each well and the plate was incubated for 3 hr at 37° in a humidified 5% CO_2 atmosphere. After the specified period, cell viability was evaluated by measurement of the absorbance at 490 nm, using a Titertek Multiskan Multisoft reader (ICN). In experiments designed to examine the protective effect of the isoprenoids on paclitaxel cytotoxicity, farnesyl-PP (1–10 μM) and geranylgeranyl-PP (0.5–7.5 μM) were added to the cell culture medium at the time paclitaxel was added and cell viability was evaluated as described above.

Assay of apoptosis in cultured cells. Internucleosomal DNA fragmentation was assayed as reported (8), with minor modifications. Briefly, cells were plated in 150-mm sterile dishes for cell culture and treated with paclitaxel (15–60 nM) for 24 hr. Additional cultures were treated with lovastatin (60 μM) as a positive control. At the end of the incubation, cells were washed with phosphate-buffered saline, pH 7.4, harvested with EDTA (1.5 mM), and pelleted by centrifugation. The cell pellet was disrupted with 0.5 ml of hypotonic lysis buffer (10 mM Tris, 1 mM EDTA, pH 7.5), containing 0.5% (v/v) Triton X-100, for 1 hr at 4°. The lysates were centrifuged at 14,000 rpm for 30 min to separate intact from fragmented chromatin, and the pellet, containing high molecular weight DNA, was discarded. The supernatant, containing apoptotic DNA, was treated with proteinase K (200 μg/ml) for 1 hr at 50° and extracted by treatment with phenol/chloroform/isoamyl alcohol (25:24:1). The DNA was then precipitated overnight at −20° in 2 volumes of 100% ethanol/0.13 M NaCl, with 20 μg of glycogen. After centrifugation at 14,000 rpm for 20 min at 4° to recover the precipitated DNA, the supernatant was discarded and the pellet was washed with 70% ethanol. Samples were resuspended in 10 mM Tris, 1 mM EDTA, pH 7.4, containing 1 mg/ml boiled bovine pancreatic RNase A, incubated for 1 hr at 50°, and finally mixed with sample buffer (5% glycerol, 10 mM EDTA, pH 8, 0.1% SDS, 0.025% bromphenol blue, 0.025% xylene cyanol). Electrophoresis was in 1% agarose gels in 40 mM Tris-acetate, 1 mM EDTA, pH 8; the bands were visualized by ethidium bromide staining and UV transillumination. A 123-bp DNA ladder was run as a standard.

Quantitative measurement of apoptosis was carried out with the Cell Death Detection enzyme-linked immunosorbent assay (Boehringer Mannheim, Indianapolis, IN), following the manufacturer's instructions. This method is based on the photometric immunoassay of cytoplasmic histone-associated DNA fragments. Cell treatment was performed as reported above, and the immunoassay was carried out with 3×10^5 cells. Results are given as enrichment factors, i.e., the specific enrichment of mono- and oligonucleosomes released by treated cells, calculated as follows: absorbance (A_{405nm}/A_{490nm}) of treated cells/absorbance (A_{405nm}/A_{490nm}) of control cells. In experiments designed to examine the protective effect of the isoprenoids on paclitaxel-induced apoptosis, farnesyl-PP (3 μM) and geranylgeranyl-PP (3 μM) were added to cell culture medium together with paclitaxel and apoptosis was evaluated as reported above.

Measurement of protein isoprenylation. PC-3 cells were plated in 60-mm culture dishes containing serum-supplemented medium and were incubated until 65% confluent. Cultures were then re-fed with fresh medium containing vehicle (0.1% dimethylsulfoxide) or paclitaxel, to give a final concentration of 15–60 nM. In each case, lovastatin (20 μM) was added to cell cultures to suppress endogenous production of mevalonic acid, thus preventing dilution of the labeled compound that penetrates the cell membrane. After an 8-hr incubation at 37°, each monolayer received (*RS*)-[5-^{3}H]mevalonolactone (100 μCi/ml), added in 100 μl of medium used for cell propagation, and incubation was continued overnight. The procedure adopted was essentially as described by James *et al.* (12). Briefly, cells were harvested with 1.5 mM EDTA and centrifuged, and the cell pellet was disrupted in lysis buffer containing 1% (v/v) Nonidet P-40, 50 mM Tris, pH 7.6, 2 mM EDTA, 100 mM NaCl, and protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 5 μg/ml pepstatin, 5 μg/ml antipain, and 5 μg/ml aprotinin), for 30 min at 4°. The detergent-soluble fraction was obtained after centrifugation for 30 min at 14,000 rpm, and an aliquot of the supernatant was taken from each sample for measurement of protein concentration, using the bicinchoninic acid reagent (13) and bovine serum albumin as a standard. A portion of each sample (100 μg) was boiled in sample buffer (50 mM Tris, pH 6.8, 100 mM dithiothreitol, 2% SDS, 10% glycerol, 0.025% bromphenol blue) and subjected to electrophoresis on a 12.5% SDS-polyacrylamide gel. Gels were stained with Coomassie blue G-250 to document equal sample loading, equilibrated for 30 min with fluorography enhancer, dried under vacuum at 80° for 2 hr, and finally exposed to Kodak X-OMAT-AR film at −70°.

Immunoprecipitation of isoprenylated proteins (p21ras and p21rap-1). To evaluate the effects of paclitaxel treatment on farnesylation and geranylgeranylation, the post-translational processing of two target proteins, p21ras and p21rap-1, was examined. The detergent-soluble protein fraction from PC-3 cells treated with paclitaxel and labeled with (*RS*)-[5-^{3}H]mevalonolactone was obtained as described above. p21ras and p21rap-1 immunoprecipitations were performed as reported (14, 15), with minor modifications. Briefly, protein concentration was adjusted to 3 mg/ml and cell extracts were mixed with 20 μl/ml levels of the anti-v-Ha-*ras* (Y13–259) or anti-*rap*-1/K-*rev*-1 (121) antibodies at 4° for 6 hr. Immune complexes were precipitated overnight at 4°, on a rotating platform, by addition of 70 μl of a 15% suspension of Protein G-Sepharose

beads in lysis buffer. The immunoprecipitates were collected by centrifugation at 14,000 rpm for 15 min at 4° and were washed four times with 1 ml each of ice-cold lysis buffer. The pellets were dissolved in 20 μl of sample buffer and the antigens were released by heating at 95° for 5 min before electrophoresis. SDS-PAGE and fluorography were performed as reported above. Gels were stained with silver stain to document that equal amounts of samples were loaded in each lane.

Data analysis. As a measure of cytotoxicity, the IC_{50} of paclitaxel was calculated by polynomial fitting of the data from cytotoxicity experiments and expressed in nanomolar units. Statistical comparisons were performed using Student's unpaired two-tailed t test. A p value of <0.05 was considered to be significant.

Results

Cytotoxicity of paclitaxel and effect of isoprenyl-PP supplementation. The effect of paclitaxel on cell viability was evaluated by the reduction of the tetrazolium salt MTS by mitochondrial dehydrogenases of metabolically active cells; the resulting soluble formazan product was measured spectrophotometrically. Treatment with paclitaxel (5–60 nM) for 24 hr produced a dose-responsive inhibition of PC-3 cell viability (Fig. 1), with a mean calculated IC_{50} of 31.2 nM. The supplementation of cell culture medium with either farnesyl-PP or geranylgeranyl-PP (3 μM) resulted in a substantial reduction of the cytotoxic effect of paclitaxel, with a significant increase in the IC_{50} (means, 48.4 and 50.5 nM, respectively; $p < 0.05$, compared with paclitaxel alone); the effect was further reduced in the presence of the combination of farnesyl-PP and geranylgeranyl-PP (3 μM) (mean IC_{50}, 55.5 nM; $p < 0.05$, compared with paclitaxel alone) (Fig. 1). Pretreatment of cells with farnesyl-PP and geranylgeranyl-PP for 24 hr before paclitaxel treatment did not improve the protective effect of the isoprenyl-PPs, whereas the use of concentrations of farnesyl-PP and geranylgeranyl-PP higher than 3 μM produced inhibition of PC-3 cell viability (data not shown).

Apoptotic cell death induced by paclitaxel. Agarose gel electrophoresis of DNA isolated from apoptotic cells demonstrated a distinctive ladder pattern consisting of multiples of a ≈180-bp subunit, as a result of nonrandom oligonucleosome-length fragmentation of DNA. After a 24-hr treatment of PC-3 cells with paclitaxel (60 nM), typical DNA laddering, indicating the occurrence of apoptosis, was clearly visible in ethidium bromide-stained gels (Fig. 2). A discrete pattern of fragmentation was also observed at the lower concentrations of 15 and 30 nM (Fig. 2). As a control, the effect of 60 μM lovastatin, an inhibitor of the mevalonic acid biosynthetic pathway that has been shown to induce apoptosis in HL-60 cells (7), was also tested. Cells treated with this drug for 24 hr manifested a pattern of 180-bp integer internucleosomal fragmentation that was less intense, compared with that produced by paclitaxel (60 nM) (Fig. 2). Similar results were obtained with the immunoassay of mono- and oligonucleosomes released into the cytoplasm of apoptotic cells after drug treatment. Exposure to paclitaxel produced a dose-responsive increase in DNA fragmentation from control levels (Fig. 3). Farnesyl-PP or geranylgeranyl-PP (3 μM each) reduced the extent of apoptotic death in PC-3 cells, whereas their combination was most effective in reducing DNA fragmentation (Fig. 3), in agreement with the data from cytotoxicity assays.

Inhibition of total cellular protein isoprenylation by paclitaxel. Radioactivity derived from the incorporation of (*RS*)-[5-^{3}H]mevalonolactone-labeled metabolites was detected in control PC-3 cells in bands corresponding to molec-

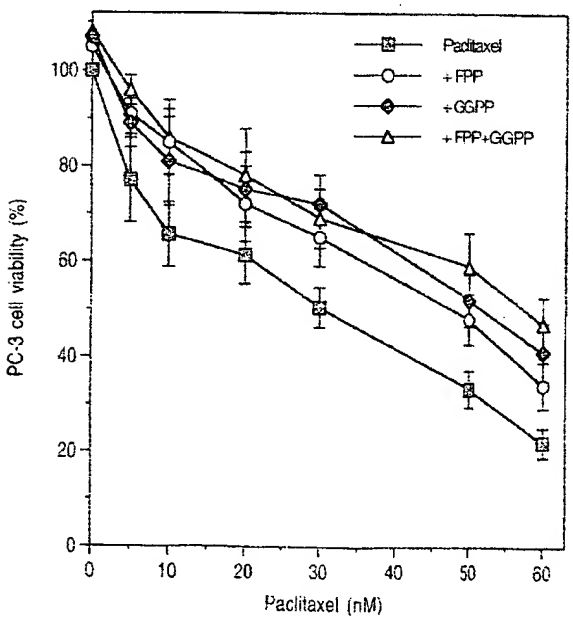

Fig. 1. Effect of 24-hr exposure to paclitaxel on PC-3 cell viability. Cells were treated with paclitaxel on day 1. To evaluate the protective activity of isoprenyl compounds, farnesyl-PP (*FPP*) and geranylgeranyl-PP (*GGPP*) (3 μM) were added simultaneously with paclitaxel. Cell viability was determined 24 hr later by measurement of the absorbance of the MTS formazan product. *Points*, mean values of triplicate experiments; *vertical bars*, standard errors.

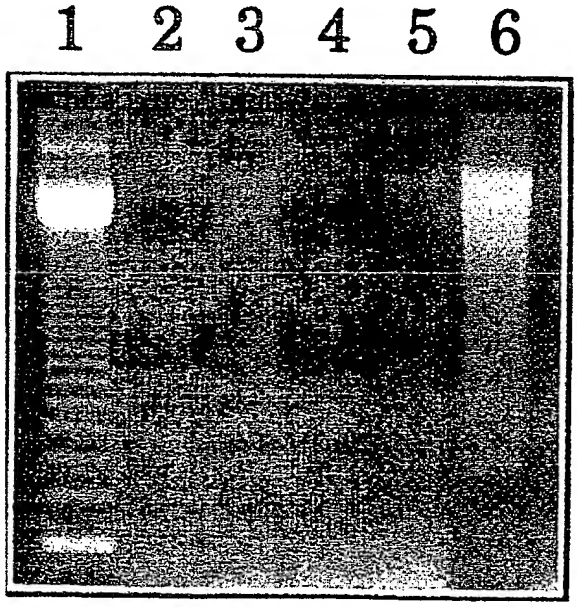

Fig. 2. Agarose gel electrophoresis of apoptotic DNA from PC-3 cells. Cells were treated with vehicle control (*lane 2*), lovastatin (60 μM) (*lane 3*), or paclitaxel at 15, 30, or 60 nM (*lanes 4–6*, respectively) on day 1. After 24 hr, cells were lysed and low molecular weight DNA was precipitated and separated by 1% agarose gel electrophoresis. DNA was visualized by ethidium bromide staining and UV transillumination. *Lane 1*, 123-bp DNA ladder.

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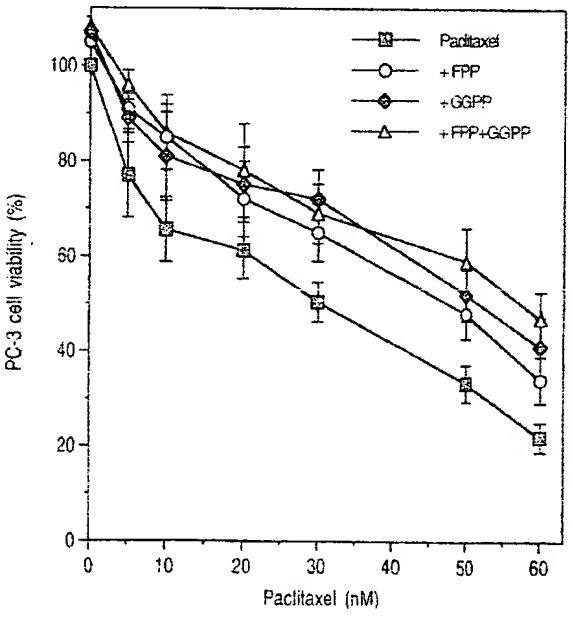

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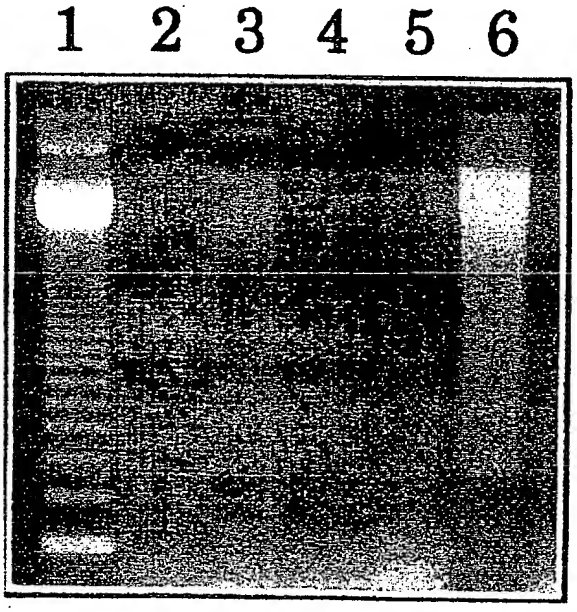

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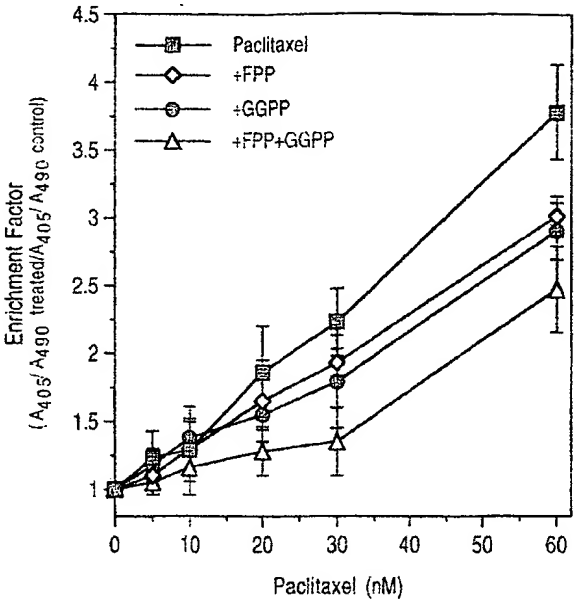

Fig. 3. Immunoassay of apoptotic DNA from PC-3 cells. Cells were treated as in Fig. 2: the production of DNA fragments was determined by photometric enzyme immunoassay and is expressed as the enrichment factor. To evaluate the protective activity of isoprenyl compounds, farnesyl-PP (*FPP*) and geranylgeranyl-PP (*GGPP*) (3 μM) were added with paclitaxel. *Points*, mean values of triplicate experiments; *vertical bars*, standard errors.

ular masses of 43, 26–21, and 17 kDa (Fig. 4), as well as at the dye front (data not shown). To test whether paclitaxel affected isoprenylation of proteins in the PC-3 cell line, extracts from cells labeled with (*RS*)-[5-^{3}H]mevalonolactone and treated with 15, 30, or 60 nM paclitaxel were resolved by SDS-PAGE and isoprenylated proteins were visualized by fluorography. Cells exhibited a substantial decrease in the intensity of the 43-, 26–21-, and 17-kDa proteins with 30 and 60 nM paclitaxel, whereas a minor reduction in the intensity of the 43-kDa protein band was observed with 15 nM paclitaxel (Fig. 4). In each experiment lovastatin, an inhibitor of HMG-CoA reductase, was used to suppress the endogenous synthesis of mevalonic acid, thus increasing the incorporation of exogenous (*RS*)-[5-^{3}H]mevalonolactone into cell proteins.

Inhibition of p21ras and p21rap-1 protein isoprenylation by paclitaxel. To determine whether paclitaxel inhibited farnesylation of p21ras and geranylgeranylation of p21rap-1, cell lysates from PC-3 cells labeled with (*RS*)-[5-^{3}H]mevalonolactone were subjected to immunoprecipitation with the anti-p21ras rat monoclonal antibody and the anti-p21rap-1 rabbit polyclonal antibody. The immunoprecipitates were then subjected to SDS-PAGE and fluorography. A single 21-kDa protein band, corresponding to prenylated p21ras (Fig. 5, *upper*) or p21rap-1 (Fig. 5, *lower*) was detected in the fluorogram of control cells. Treatment of PC-3 cells with paclitaxel produced a substantial reduction in the incorporation of the label into immunoprecipitable p21ras and p21rap-1 proteins at 30 and 60 nM (Fig. 5), whereas a modest inhibition was detected at 15 nM in the case of p21rap1 (Fig. 5). These results were in agreement with the findings from (*RS*)-[5-^{3}H]mevalonolactone-labeled total cellular proteins reported above.

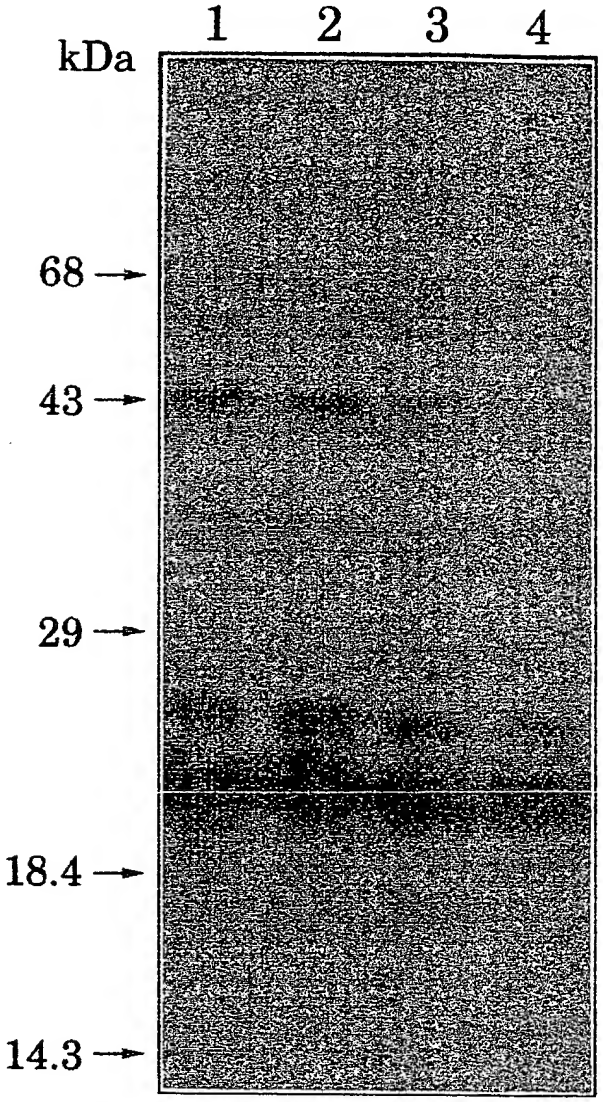

Fig. 4. Inhibition of protein isoprenylation by paclitaxel. Cells were treated with vehicle (*lane 1*) or paclitaxel at 15, 30, or 60 nM (*lanes 2–4*, respectively) on day 1, and after 8 hr cells were labeled with (*RS*)-[5-^{3}H]mevalonolactone (100 μCi/ml). After 18 hr cells were harvested and detergent-soluble extracts were obtained. Samples were analyzed by SDS-PAGE, and proteins were visualized by fluorography for 5 days at −70°. *Numbers on the left*, molecular mass (in kDa).

Discussion

Prenylation of proteins by intermediates of the isoprenoid biosynthetic pathway represents a newly discovered type of post-translational modification (16). The characterization of protein prenylation biology and enzymology has opened up areas for the development of inhibitors that can modify physiological processes involved in the control of cell proliferation. The experiments reported here provide the first evidence for isoprenylation inhibition of a subset of PC-3 cell proteins, including p21ras, by the anticancer drug paclitaxel, a highly lipophilic diterpene compound isolated from the bark of *T. brevifolia*. Furthermore, this effect was demonstrated at 30 nM paclitaxel, within the therapeutic range of drug levels and close to the IC_{50} for PC-3 prostate cancer cells.

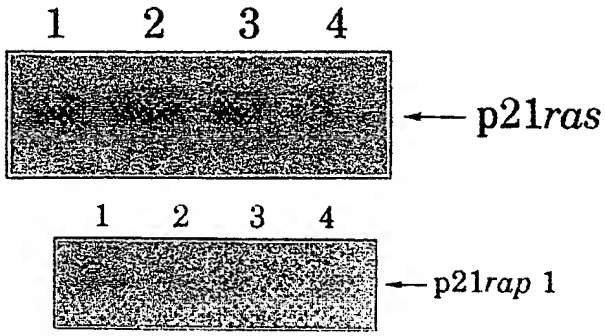

Fig. 5. Inhibition of farnesylation of p21ras (*upper*) and geranylgeranylation of p21rap-1 (*lower*) by paclitaxel. Cells were treated with vehicle (*lane 1*) or paclitaxel at 15, 30, or 60 nM (*lanes 2-4*, respectively) on day 1, and 8 hr later cells were labeled with (*RS*)-[5-^{3}H]mevalonolactone (100 μCi/ml). After an additional 18 hr, cells were harvested and detergent-soluble extracts were obtained. Lysates were immunoprecipitated with anti-p21ras or anti-p21rap-1 antibodies and resolved by SDS-PAGE, and proteins were visualized by fluorography for 10 days (p21ras) or 21 days (p21rap-1) at −70°.

Paclitaxel has demonstrated remarkable antitumor activity *in vitro* and *in vivo* (9, 17) and is currently used for the treatment of metastatic breast cancer (18) and platinum-resistant ovarian cancer (19). The drug is a novel mitotic inhibitor that promotes microtubule assembly and stabilizes microtubules at concentrations as low as 50 nM *in vitro* (9). This is in contrast to the mechanism of action of *Vinca* alkaloids and podophyllotoxins, which induce microtubule disassembly. Since the pioneering work of Manfredi *et al.* (20), the microtubule-binding effect of paclitaxel has been reported to be specific, saturable, and correlated with the cell growth inhibitory effect.

However, basic questions regarding the pharmacodynamics of paclitaxel persist; in particular, the mechanism of drug-induced apoptotic cell death remains to be fully elucidated. The drug is able to induce DNA laddering in the human ovarian cancer cell line OVCAR-3 (21) and in the human myeloid leukemia cell line HL-60 (8), but only after prolonged exposure (48 hr) or at higher concentrations in the culture medium (0.1–1 μM) than those adopted in the present study. One possible explanation for this finding is that the PC-3 cell line is particularly sensitive to the induction of DNA cleavage by paclitaxel; in addition, the number of cells used in previous studies for apoptosis experiments (8, 21) was smaller than the number processed in the present investigation. Cell death by apoptosis occurs in many physiological and pathological settings, but the recent report that the inhibition of isoprenoid biosynthesis triggers apoptosis in HL-60 cells (7) links apoptotic death to the effect of a new class of drugs affecting cell proliferation, of which lovastatin represents the first generation.

It has been recently proposed that DNA fragmentation results from the loss of compartmentalization of DNase I, which would reach the nucleus due to the breakdown of the endoplasmic reticulum and the nuclear membrane (22). It is known that isoprenylated proteins play a crucial role in maintenance of the integrity of these structures, in particular lamins A and B (23), which form the nuclear lamina, and low molecular weight G proteins, including p21rho, which regulate cytoskeletal functions (15, 24, 25). Moreover, it has been shown that inhibition of isoprenylation impairs the association of lamins with the nuclear membrane (23) and induces actin depolymerization and alteration of cell morphology (10), indicating that prenylated proteins play a critical role in regulating the state of intracellular actin. Suppression of the mevalonate pathway in HL-60 cells blocked the membrane association of lamin A as a result of the inhibition of its prenylation, inasmuch as the membrane association of lamin C, which does not require isoprenylation, remained unaltered (7). Thus, paclitaxel-induced DNA fragmentation could be dependent, at least in part, on the breakdown of the nuclear membrane and the occurrence of cytoskeletal alterations, resulting from the inhibition of lamin processing and G protein prenylation, respectively. Additional mechanisms of drug-induced apoptosis include the loss of Bcl-2 protein during the prolonged mitotic arrest (21) or decreased expression of the *bcl-2* gene (8), which is known to protect cells from undergoing apoptosis.

Chemically, paclitaxel is a terpene; other compounds of this class have been previously shown to produce inhibition of protein prenylation. *d*-Limonene, a plant monoterpene resulting from the cyclization of geranyl-PP, has chemotherapeutic activity against chemically induced rat mammary cancer, in which *ras* genes are frequently activated (26). The inhibition by *d*-limonene of protein isoprenylation in NIH/3T3 fibroblasts was obtained at 0.5–5 mM (14), a range of concentrations higher than those used for paclitaxel in this report. Furthermore, the farnesol analogue farnesylamine has been shown to inhibit the growth of *ras*-transformed NIH/3T3 cells and the prenylation of cellular proteins, including p21ras, by inhibition of the farnesyl-protein transferase activity (27). However, paclitaxel appears to be much more potent at inhibiting isoprenylation than is any other tested terpene.

In the present study, the choice of p21ras and p21rap-1 to study the specific effects of paclitaxel on the post-translational processing of cellular proteins was dependent not on their biological role but, rather, on the fact that they undergo differential isoprenylation, namely farnesylation (p21ras) and geranylgeranylation (p21rap-1). Paclitaxel did not interfere with the production of p21ras and p21rap-1, as demonstrated by silver staining of the immunoprecipitated material in polyacrylamide gels, but did interfere with the biochemical modifications essential to their localization. It is unlikely that the drug inhibits the enzyme HMG-CoA reductase, because 20 μM lovastatin was present throughout the assays and (*RS*)-[5-^{3}H]mevalonolactone was used to label cell proteins. In agreement with this finding, mevalonic acid was unable to prevent paclitaxel toxicity in the PC-3 cell line.

We have recently demonstrated that proteins can be labeled by exposure of lovastatin-treated PC-3 cells to [^{3}H]farnesyl-PP and [^{3}H]geranylgeranyl-PP (28). Thus, acyclic isoprenoids are incorporated into cell proteins and are able to restore prenylation while HMG-CoA reductase activity is suppressed. The protective effects of farnesyl-PP and geranylgeranyl-PP were evaluated under different conditions, including preincubation for 24 hr or extended treatment for 48 hr. Under no circumstances was a better protection, compared with coincubation, observed. We do not have experimental data to explain why equal levels of rescue were obtained with farnesyl-PP and geranylgeranyl-PP, even though geranylgeranylated proteins are predominant in the cells. One possible explanation is that not only the absolute

amounts of proteins but also their biological roles are critical. Thus, the problem is more complex than the quantitative approach would suggest. The protective effect of the isoprenoids was not improved by increasing their concentrations up to 6 μM, whereas inhibition of proliferation was obtained at concentrations higher than 8 μM. The mechanism of the detrimental effects of high concentrations of the allylic isoprenols farnesyl-PP and geranylgeranyl-PP on cell growth is not clear. We suggest that their hydrophobic structures facilitate the interaction with cell membranes and produce alterations of their biophysical properties. In addition, an excess of intracellular isoprenoids could itself produce feedback inhibition of the mevalonate pathway.

The results of the present study suggest that the site of paclitaxel activity is distal to the synthesis of mevalonic acid. Thus, possible targets are either the enzymes involved in the synthesis of isoprenoids, including farnesyl- and geranylgeranyl-PP, or the activity of prenyl-protein transferases, including farnesyl-protein transferase and geranylgeranyl-protein transferase types I and II (1, 16). Further work will be required to define the specific site of drug action; in particular, the analysis of cellular levels of nonsterol isoprenoids upon treatment with paclitaxel could provide possible clues to answer this question.

At variance with lovastatin, whose toxic effect can be overcome by the simultaneous addition of either mevalonate or farnesol (29, 30), the lack of total prevention of paclitaxel cytotoxicity by supplementation with farnesyl-PP and geranylgeranyl-PP is not unexpected and is likely to be dependent on the well known microtubule-binding property of paclitaxel.

Finally, the inhibition of p21ras farnesylation in PC-3 prostate cancer cells by paclitaxel causes nonprenylated *ras* to accumulate in the cytosol; the biological consequence of this could be relevant in the overall effect of the drug and deserves further investigation.

In summary, the data presented in this report demonstrate that (i) paclitaxel inhibits the farnesylation and geranylgeranylation of growth control-associated proteins at concentrations within the therapeutic range and (ii) the resulting cytotoxic effect is partially prevented by supplementation with two polyisoprenyl PPs, farnesyl-PP and geranylgeranyl-PP. This novel pharmacodynamic effect of paclitaxel may play a significant role in the overall chemotherapeutic activity of the drug in the treatment of cancer.

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